Remote MR Sensing of pH and Cell Viability using LipoCEST-filled Microcapsules

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Introduction

Cell therapy has been applied to a variety of degenerative disorders, including Type I diabetes, where pancreatic islet transplantation allows fine regulation of insulin. The site and function of transplanted cells are important parameters for assessing the success of therapy. Thus, new biomaterials are required not only to image but also to detect the viability of cells *in vivo*. Cell tracking of transplanted cells has been achieved via various methods including the use of MR contrast agents[1,2]. However, the imaging of cell viability remains a challenge. Subtle changes in physiological pH are closely related to cell activities, such as apoptosis and insulin secretion. We introduce the use of LipoCEST-enhanced microcapsules for visualization and monitoring of the viability of transplanted cells, and at the same time provide immunoprotection. These microcapsules are designed to sense pH changes in the extracellular environment, enabling us to monitor cell viability.

Materials&Methods: Liposomes consisting of phosphatidyl choline, NBD (7-nitro-2-1,3-benzoxadiazol-4-yl)-methyl]amino]), cholesterol, DSPE-PEG2000, L-arginine, and rhodamine were used as LipoCEST agents[3]. Pancreatic islet cells and murine βTC6 insulinoma cells were cultured in standard medium, and were co-encapsulated with liposomes in alginate-PS-alginate microcapsules[5]. As a proof-of-concept, non-viable cells were included through osmotic lysis, and cell viability was determined by fluorescence microscopy using vital dyes. CEST images were acquired at 310 K using a 11.7 T Bruker Avance system equipped with a 15-mm birdcage RF coil. A slice thickness of 0.5 mm was used. Typical image readout settings were: acquisition bandwidth =50 kHz, T_R=6.0 sec, T_E= 4.3 ms and RARE factor =16. The field of view was 13×13 mm, resulting in an in-plane resolution of 101×101 μm with an acquisition matrix size of 128×128. A continuous wave (CW) RF saturation pulse was used in the magnetization transfer module in all cases. The saturation offset was swept from -5 ppm to +5 ppm with a 0.2 ppm increment, $\omega_1 = 4.7$ μT (200 Hz), $t_{sat} = 4$ sec. Images were collected including a WASSR image set with reduced T_R to determine the B_0 shifts.[28] In a pixel-wise manner, the WASSR z-spectrum was analyzed using a maximum symmetry algorithm to find the frequency offset ($\Delta\omega_{sw}$) at where the strongest water attenuation occurs. The maximal symmetry algorithm was used because in theory, the direct water saturation is symmetric around the water resonant frequency, and its shape will not be affected by B_0 inhomogeneity. Upon obtaining the WASSR B_0 map, the frequency of each pixel of all CEST images was corrected. . $MTR_{asym} = (S^{-\Delta\omega} - S^{+\Delta\omega})/ S^{-\Delta\omega}$ was computed to quantify the CEST contrast in the studies.

Results&Discussion: As shown in Fig. 1, LipoCEST microcapsules displayed a pH-dependent CEST contrast, with the MTR_{asym} changing by 80% at pH 6-7.5 (Fig. 1). Microcapsules could be imaged by both fluorescence and CEST imaging. Fig. 2A shows the fluorescence images of LipoCEST microcapsules, which contains βTC6 insulinoma cells. Live vs. dead insulinoma cells were stained with Hoechst and propidium iodide (dead cells) to display cell viability. These live and dead cell microcapsules were used to demonstrate the difference in CEST contrast *in vitro*. Fig. 2B shows the MTR_{asym} map of these microcapsules with a significant difference in contrast ($p<0.01$). The live cells had a higher MTR_{asym}, than that of dead-cell model, which corresponds to a decrease in pH. This supports our hypothesis that dead cells induce a lowering of the pH. In addition, the viability of pancreatic islet cells and murine βTC6 insulinoma cells remained >90% at three days post-encapsulation (Fig. 3). This *in vitro* study clearly showed that the viability of encapsulated cells can be monitored by this LipoCEST microcapsules safely.

Conclusions: The CEST contrast produced by LipoCEST microcapsules is dependent on pH changes which will be induced to change through variations in cell viability. These capsules are the first demonstration of the ability of CEST to monitor the viability of encapsulated cells. As CEST imaging can be used clinically without the use of metal-based contrast agents, our approach may facilitate future monitoring of the functionality of pancreatic islet cell transplants in diabetic patients.

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References: [1] Lu Y. et al. Proc. Natl. Acad. Sci. USA. 103(30):11294-9 (2006). [2] Evgenov NV. et al. Nat. Med. 21(1): 144-8 (2006); [3] McMahon MT. et al. Magn. Reson. Med. 60(5): 803-12 (2008). [4] Barnet BP. et al. Nat. Med. 13(8): 986-91 (2007).

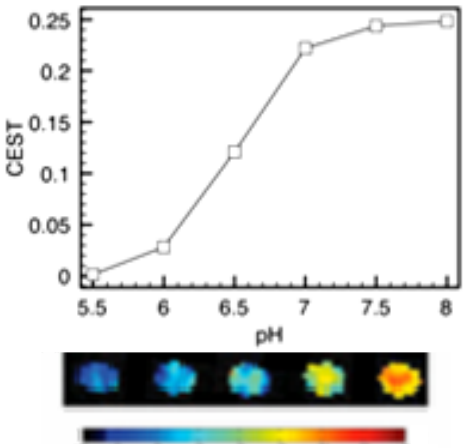

Fig. 1 Dependence of CEST contrast (MTR_{asym}) on pH for LipoCEST microcapsules (Top); MTR_{asym} map at pH 5.5, 6.0, 6.5, 7.0, 7.5 (Bottom).

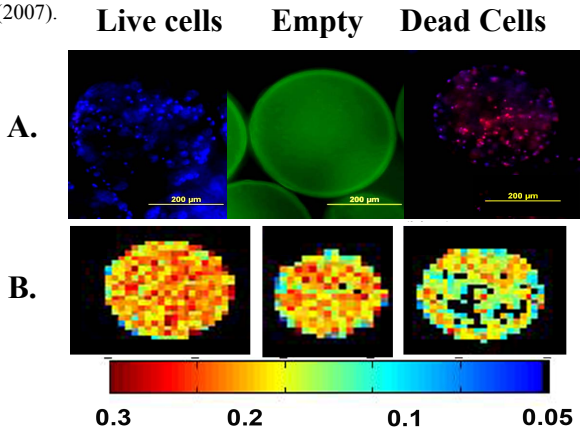

Fig. 2 (A) Fluorescence image of LipoCEST microcapsules containing live, no cell (Empty) and dead murine βTC6 insulinoma cells stained by Hoechst and PI; (B) Their respective MTR_{asym} map.

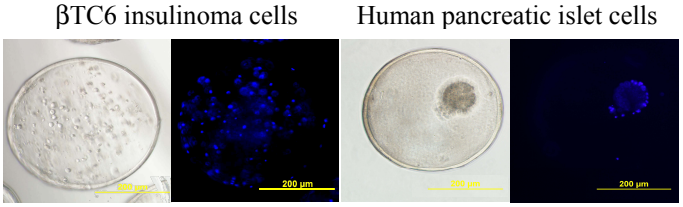

Fig. 3 Light microscope and fluorescence images of LipoCEST microcapsules with murine insulinoma cells and human pancreatic islet cells at 3 days post-encapsulation stained by Hoechst and PI.